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qPCR QUANTIFICATION OF AMMONIA OXIDIZING BACTERIA: WHAT SHOULD THE TARGET BE?

Sanin Musovic, Alejandro Palomo, Vaibhav Diwan, Arnaud Dechesne and Barth F. Smets

Department of Environmental Engineering, Technical University of Denmark

Ammonia oxidizing bacteria (AOB) perform the first step of nitrification, a key step in the Nitrogen cycle in both natural and engineered systems. In addition to their well-known role in wastewater treatment, they are also essential in rapid sand filter at waterworks treating anaerobic groundwater for drinking water production. Being able to quantify precisely the abundance of this functional group is thus important to be able monitor these processes.

AOB are moderately diverse Beta-Proteobacteria that all carry the *amoA* gene coding for the ammonia monooxigenase. Therefore, molecular quantification can be carried out by targeting either the 16S rRNA gene or *amoA*, for which standard primer sets are widely used. Using these two approaches to quantify AOB abundance across three Danish rapid sand filters (RSFs) revealed a significant discrepancy: in two RSFs, the *amoA*-based qPCR consistently yielded estimate ~50 fold lower than that obtained with the 16S one. We carried out cloning sequencing and coverage analysis of the primer sets to explain this observation. Result showed that the primer sets have an adequate specificity but differ in their coverage. *In silico* analysis indicated that the *amoA* primer set has a narrower coverage than the 16S rRNA one and thus led to an underestimation of AOB in RSFs hosting broad AOB diversity. This highlights the importance of the choice of primer set to quantify functional groups in environmental samples.